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Publication Title:

ANTI-OBESITY DRUGS

Abstract:

Obesity is treated by the administration to a subject of a compound having the general formula (I): R4-(CH2)n-CO-N(R1)-CH(R2)-CO(-R3), wherein R1 represents H or CH3; R2 represents a side chain of a naturally occurring amino acid; R3 represents OH, OCH2CH3 and NH2; n is 6-18; and R4 represents CH3 or a group having the general formula (II): R3-CO-CH(R2)-N(R1)-CO-, wherein R1, R2 and R3 have the above meanings. The compounds of formula (I) wherein R4 is a group of formula (II), are novel compounds.

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(54) Title: ANTI-OBESITY DRUGS

(57) Abstract

Obesity is treated by the administration to a subject of a compound having the general formula (I): R_4-$(CH_2)_n$-CO-$N(R_1)$-$CH(R_2)$-$CO(-R_3)$, wherein R_1 represents H or CH_3; R_2 represents a side chain of a naturally occurring amino acid; R_3 represents OH, OCH_2CH_3 and NH_2; n is 6-18; and R_4 represents CH_3 or a group having the general formula (II): R_3-CO-$CH(R_2)$-$N(R_1)$-CO-, wherein R_1, R_2 and R_3 have the above meanings. The compounds of formula (I) wherein R_4 is a group of formula (II), are novel compounds.

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ANTI-OBESITY DRUGS

FIELD OF THE INVENTION

The present invention is in the field of treatment and prevention of obesity. The present invention provides compositions and methods for the treatment or prevention of usch disorders utilizing, as active ingredient, lypophilic derivatives of natural amino acids. Furthermore, the present invention provides certain such novel compounds.

PRIOR ART

The following prior art is believed to be relevant as a background to the present invention:

1. Bar-Tana *et al., J. Biol. Chem.,* 1985, 260, 8404-8410.
2. Rose-Kahn *et al., J. Biol. Chem.,* 1985, 260, 8411-8415.
3. Bar-Tana *et al., J. Lipid Res.,* 1988, 29, 431-441.
4. Frenkel *et al., J. Biol. Chem.,* 1988, 263, 8491-8497.
5. Tzur *et al., Diabetes,* 1988, 37, 1618-1624.
6. U.S. Patent No. 4,634,795.
7. U.S. Patent No. 4,689,344.
8. U.S. Patent No. 4,711,896.
9. U.S. Patent No. 4,908,385.

In the following text, reference to these prior art publications will be made by indicating in brackets their number from the above list.

BACKGROUND OF THE INVENTION

Energy from food is primarily provided by carbohydrates and lipids. Carbohydrates usually supply the immediate energy needs and their excess is stored as glycogen in the liver or converted to lipids. Lipids can also be metabolized as immediate energy providing substances but their rate of energy provision is relatively slow and they are generally stored in the body for use in states of deprivation. Lipid is stored in the body mostly as fat under the skin and consumption of lipids and carbohydrates beyond the metabolic need leads to fattening. The associated medical and aesthetic problems, are a major concern in modern society.

Apart from surgery and dietary means for reduction of fat absorption in the small intestine, there are presently no satisfactory means for reducing fat storage in the body and the current means of choice are still diet and exercise. There is, however, a desire for drugs which will reduce fat accumulation by inhibiting lipid and lipoprotein synthesis in the liver. Recently, a series of ß,ß′ tetramethyl substituted α,ω dicarboxylic acids (MEDICA) have been synthesized and suggested as potential anti-fattening drugs[1-4,6-9]. The most potent drug of this series was found to be the hexadecane derivative (MEDICA 16). It was demonstrated that MEDICA, which is a non-naturally occurring fatty acid, could inhibit biosynthetic pathways of triglycerides and cholesterol in the liver. Experiments with MEDICA 16 given in the diet to normal and obese rats have indicated a strong inhibition of glyceride and cholesterol biosynthesis in the liver evidenced by a marked reduction in their serum contents[3]. Furthermore, in the obese animals adipose tissue was reduced by about 75% over the whole body concomitantly to extensive weight loss[5]. However, the metabolic

clearance of these compounds via integration into glycerol esters or via oxidation is relatively slow due to the presence of carboxylate at the two edges of the molecules and the ß alkyl substitution. Despite their impressive effect, MEDICA are expected to exert a long term toxicity due to their non-compatible molecular structure. Thus, chronic intake of MEDICA, which is required for maintaining a low fat state, would likely be associated with adverse toxic effects in the long run.

OBJECTS OF THE INVENTION

It is the object of the present invention to provide a pharmaceutical composition, method and dietary supplements for the treatment and/or prevention of obesity. More specifically, it is an object of the present invention to provide such composition and method utilizing lipophilic derivatives of natural amino acids.

It is a further object of the present invention to provide certain novel lipophilic derivatives of natural amino acids useful in such compositions and methods.

The remaining objects of the present invention will be illustrated from the following description and claims.

GENERAL DESCRIPTION OF THE INVENTION

By a first of its aspects, the present invention provides a pharmaceutical composition for the treatment of obesity comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound having the general Formula I:

$$R_4-(CH_2)_n-CO-N(R_1)-CH(R_2)-CO(R_3) \qquad (I)$$

wherein R_1 represents H or CH_3;

R_2 represents a side chain of a naturally occurring amino acid;

R_3 represents OH, OCH_2CH_3 and NH_2;

n is 6 – 18, preferably 12 – 16; and

R_4 represents CH_3 or a group having the general Formula II:

$$R_3–CO–CH(R_2)–N(R_1)–CO– \quad (II)$$

wherein R_1, R_2 and R_3 have the above meanings.

The present invention also provides methods for the treatment of obesity comprising administering to a subject in need an effective amount of an active ingredient being a compound having the general Formula I as defined above.

The present invention also provides a dietary supplement for the prevention of obesity comprising an active ingredient being a compound of the general Formula I as defined above.

The compounds according to Formula I wherein R_4 represents a group of Formula II are novel, and such compounds are also provided by the present invention.

An example of compounds of Formula I wherein R_4 is CH_3, is N–palmitoyl sarcosine (P–Sar) having the Formula III:

$$CH_3–(CH_2)_{14}–CO–N(CH_3)–CH_2COOH \quad (III)$$

Examples of compounds of Formula I, in which R_4 represents a group having the general Formula II, are N,N′ sebacoyl bis–glycine (GSG), having the Formul IV:

$$HOOC–CH_2–NH–CO–(CH_2)_8–CO–NH–CH_2–COOH \quad (IV)$$

N,N′ sebacoyl bis ℓ–aspargin (NSN), having the Formula V:

$$(CH_2)_8–[CO–NH–CH(CH_2CONH_2)–COOH]_2 \quad (V)$$

N,N′ sebacoyl bis–sarcosine [S(Sar)$_2$] having the Formula VI:

$$(CH_2)_8–[CO–N(CH_3)–CH_2–COOH]_2 \quad (VI)$$

N,N′ sebacoyl bis–sarcosine–ethylester [S(SarOEt)$_2$], having the Formula VII:

$$(CH_2)_8–[CO–N(CH_3)–CH_2–COO–C_2H_5]_2 \quad (VII)$$

and

N,N′ sebacoyl bis–phenylalanine (FSF), having the Formula VIII:

$$(CH_2)_8–[CO–NH–CH(CH_2–C_5h_6)–COOH]_2. \quad (VIII)$$

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the effect of NSN (0.1% w/w in purina) on weight of 3 months old mice during a 1 month *ad lib.* feeding;

Fig. 2 shows the effect of several anti–fattening agents including a fattening diet in accordance with the invention (0.1% w/w in purina + 6% corn oil) on weight of 3 months old mice;

Fig. 3 shows the daily weight of adult mice administered with various anti–fattening agents of the invention; and

Fig. 4 shows the effect of treatment with NSN on the ^3H_2O incorporation to liver and adipose tissue of adult mice.

The present invention will now be described with reference to the following non-limiting examples.

Example 1: preparation of compounds

Lypophilic derivatives of the natural amino acids can be synthesized via the N-hydroxy succinimide ester of the respective fatty acid. Such active esters react with primary or secondary amine to form the corresponding amide while liberating a free N-hydroxy succinimide.

In the following are three examples of such synthesis:

A: preparation of P-Sar

Hydroxy succinimide ester of palmitic acid (PHS) was obtained from SIGMA. One volume of 30mM PHS in tetrahydrofuran was mixed with one volume of excess sarcosine (0.3 M) in 0.1 M of aqueous sodium bicarbonate according to the procedure described by Lapidot *et al., J. Lipids Res.*, 1967, 8, 142. The mixture was mixed at 40°C for 24 hours. The tetrahydrofuran was then evaporated and the mixture was acidified to pH 1 with HCl, whereupon the crude product precipitated and was collected. After washing with water the product was crystallized from isopropanol.

B: preparation of GSG

(a) 1 mole of sebacic acid was reacted with 2 moles of N-hydroxysuccinimide and 2 moles of dicyclohexyl carbondiimide in ethyl acetate. The resulting compound, 1,10 sebacoyl di (N-hydroxy-succinimide) ester, [Seb-$(NHS)_2$] was crystallized from isopropanol. Seb$(NHS)_2$ was found to have an m.p. of 159°C.

(b) 1 volume of 30mM Seb$(NHS)_2$ in tetrahydrofuran with 1 volume excess glycine (0.3 M) in aqueous 0.1 M sodium bicarbonate

according to the procedure described by Lapidot *et al., supra*. After 24 hours of mixing at 40°C, the tetrahydrofuran was evaporated under reduced pressure and the product was precipitated by acidifying with 1M HCl to pH 1. The precipitate was collected and washed with water. Crystallization was from isopropanol.

C: preparation of NSN

NSN was prepared by the same procedure of Example (B) except that instead of glycine in step (b), l–asparagine was used.

The remaining compounds described in the following were prepared in a similar manner, *mutatis mutandis*.

Example 2: Experimental results

(a) Two groups of 5 three months old mice were fed with purina, *ad lib*. The diet of 1 group was supplemented with NSN (0.1% w/w in purina). The weight increase of the mice in each group was measured over 31 days and the results shown in Fig. 1 clearly demonstrate that the weight increase of the experimental group, was far less than that in the control group.

(b) 5 groups of 5 three months old mice each, were fed with a fattening diet consisting of purina and 6% corn oil, and out of these five groups, the diet of four was supplemented with anti–fattening drugs in accordance with the invention (0.1% w/w in the food). The following drugs were tested: P–Sar, GSG, $S(Sar)_2$ and $S(SarOEt)_2$.

The results shown in Fig. 2 clearly demonstrate that the weight increase of the treated animals was far less than that of the animals of the control group.

(c) Adult mice were divided into 9 groups of five mice each, and were fed with normal purina *ad lib.* and 8 groups received one of the following supplements in their diet (0.25% or 0.35% w/w in the food): dNSN, NSN, dlFSF and FSF.

One group did not receive any supplement and served as control.

Food consumption was *ad lib.*

The treatment was over a period of 40 days after which it was ceased and all groups of animals returned to a normal diet.

The results shown in Fig. 3 clearly demonstrate that some of the supplements caused even a slight increase over control. Thus for example, while an increase was observed with 0.25% FSF, a considerable decrease in weight over the entire tested period was observed with 0.35%. FSF. Accordingly it is believed that upon increase of the concentration of these drugs they will all have an anti-fattening affect.

(d) 8-12 months old mice were administered with NSN either intra peritonelly (I.P.) or Per Os (P.O.), 10 mg per day for 4 days. The incorporation of ^3H_2O to liver and adipose tissue was tested. For that purpose tritiated water was injected I.P. after overnight fast and 2 hours later the animals were sacrificed and the incorporation into lipids of the liver and the adipose tissue were determined by measuring radioactivity.

The results shown in Fig. 4, clearly demonstrate the decrease in treated water incorporation into the treated animals.

CLAIMS:

1. A pharmaceutical composition for the treatment of obesity comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound having the general Formula I:

$$R_4-(CH_2)_n-CO-N(R_1)-CH(R_2)-CO(-R_3) \quad (I)$$

wherein R_1 represents H or CH_3;

R_2 represents a side chain of a naturally occurring amino acid;

R_3 represents OH, OCH_2CH_3 and NH_2;

n is 6 – 18; and

R_4 represents CH_3 or a group having the general Formula II:

$$R_3-CO-CH(R_2)-N(R_1)-CO- \quad (II)$$

wherein R_1, R_2 and R_3 have the above meanings.

2. A pharmaceutical composition according to Claim 1, comprising as active ingredient a compound which is a member of the group consisting of N-palmitoyl sarcosin, N,N'-sebacoyl bis-glycine, N',N-sebacoyl bis ℓ-aspargine, N,N'-sebacoyl bis d-aspargine N',N-sebacoyl bis sarcosin, N,N'-sebacoyl bis-sarcosin-ethylester and N,N'-sebacoyl bis-phenylalanine.

3. A dietary supplement for preventing obesity comprising a compound of formula I as defined in Claim 1.

4. A method for the treatment of obesity comprising administering to a subject in need an effective amount of a compound of formula I as defined in Claim 1.

5. Use of a compound having the general formula I as defined in claims 1 or 2 for the preparation of a pharmaceutical composition for the treatment of obesity.

6. A compound of the general formula I:

$$R_4-(CH_2)_n-CO-N(R_1)-CH(R_2)-CO(R_3) \qquad (I)$$

wherein R_1 represents H or CH_3;

R_2 represents a side chain of a naturally occurring amino acid;

R_3 represents OH, OCH_2CH_3 and NH_2;

n is 6 – 18; and

R_4 represents a group of the general formula II:

$$R_3-CO-CH(R_2)-N(R_1)-CO- \qquad (II)$$

wherein R_1, R_2 and R_3 have the above meanings.

7. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound according to claim 6.

8. Use of a compound having the general Formula I:

$$R_4-(CH_2)_n-CO-N(R_1)-CH(R_2)-CO(-R_3) \qquad (I)$$

wherein R_1 represents H or CH_3;

R_2 represents a side chain of a naturally occurring amino acid;

R_3 represents OH, OCH_2CH_3 and NH_2;

n is 6 – 18; and

R_4 represents CH_3 or a group having the general Formula II:

$$R_3-CO-CH(R_2)-N(R_1)-CO- \qquad (II)$$

wherein R_1, R_2 and R_3 have the above meanings for combatting obesity.

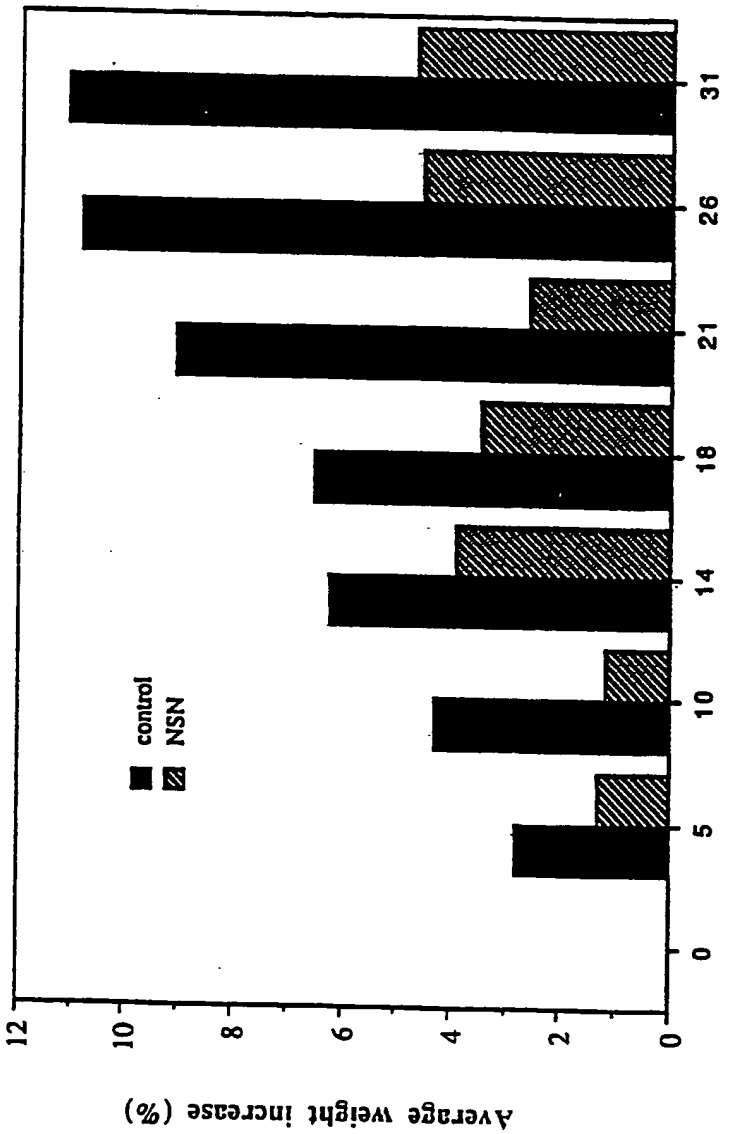
Average weight increase (%)
0
2
4
6
8
10
12
0
5
10
14
18
21
26
31
day
control
NSN

Figure 1

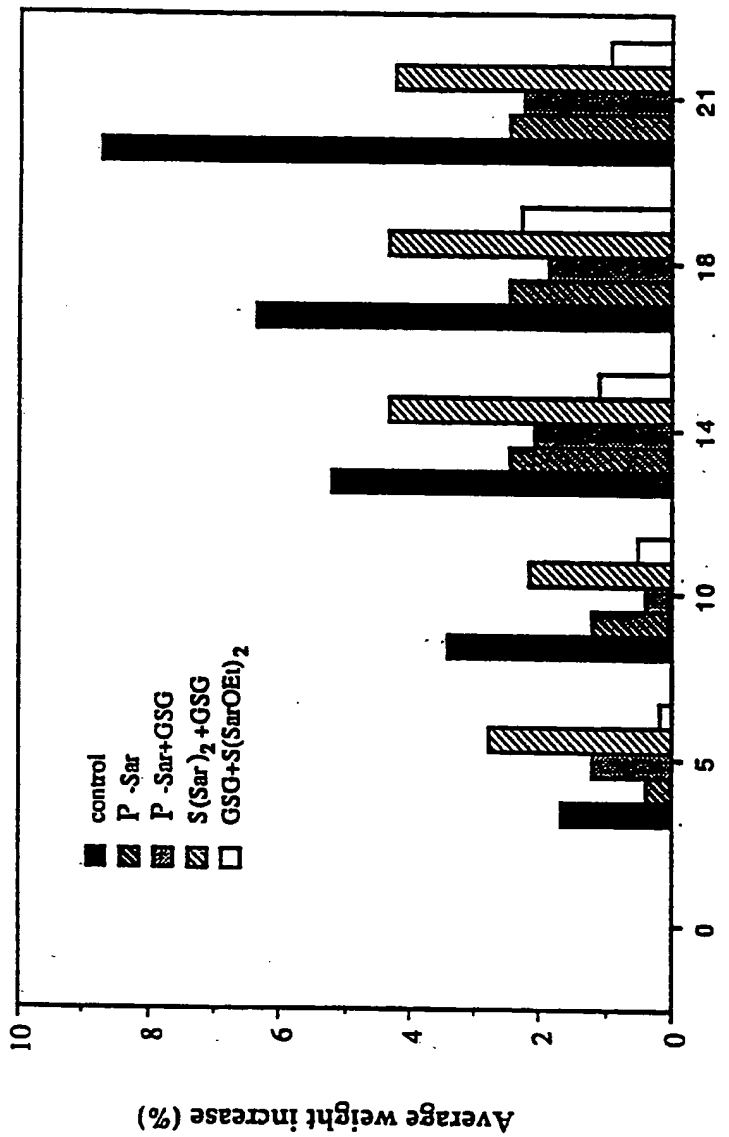
Average weight increase (%)
0
2
4
6
8
10
0
5
10
14
18
21
day
control
P -Sar
P -Sar+GSG
S(Sar)2+GSG
GSG+S(SarOEt)2

Figure 2:

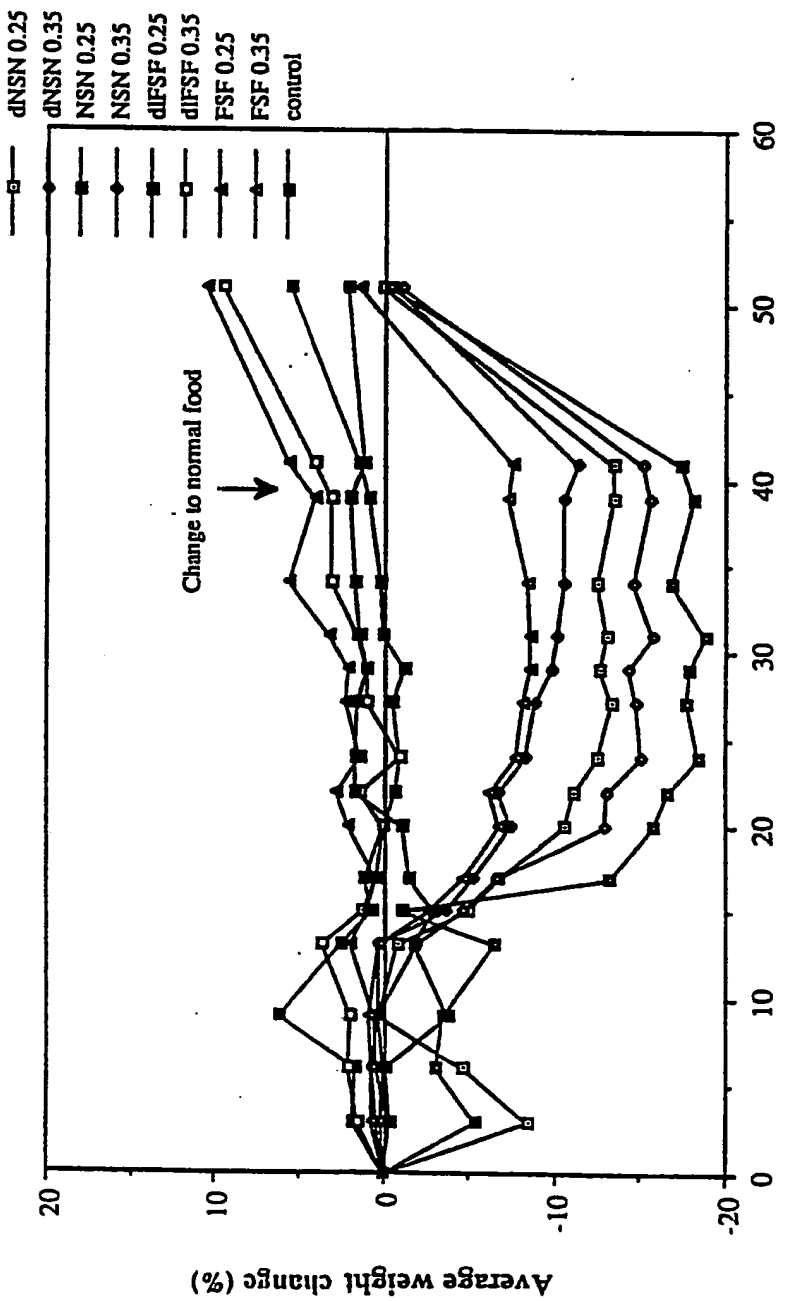
dNSN 0.25
dNSN 0.35
NSN 0.25
NSN 0.35
dIFSF 0.25
dIFSF 0.35
FSF 0.25
FSF 0.35
control
Change to normal food
Average weight change (%)
day
20
10
0
-10
-20
0
10
20
30
40
50
60

Figure 3:

Incorporation of tritiated water
(% of control)
120
100
80
60
40
20
0
I.P.
P.O.
control
liver
adipose
control
liver
adipose

Figure 4:

INTERNATIONAL SEARCH REPORT

PCT/EP 93/01014

International Application

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K31/225; A61K31/195; C07C233/47; C07C237/22

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K ; C07C

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	DE,A,2 234 399 (DR. KARL THOMAE GMBH) 31 January 1974 see page 9 see claims 1,2 ---	1,2
X	J. CHEM. SOC., CHEM. COMMUN. no. 9, 1986, pages 659 - 661 R.T.C. BROWNLEE 'The synthesis and characterization of a series of bis-intercalating bis-anthracyclines' see page 659 --- -/--	6

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
30 JULY 1993	11. 08. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	KRAUTBAUER B.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED T RELEVANT (CONTINUED FROM THE SECOND SHE

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	KHIM.-FARM. ZH. vol. 26, no. 2, 1992, pages 43 - 45 N.A. GRIGORYAN 'Synthesis and antistaphylococcal activity of dicarboxylic acid derivatives containing an amino acid fragment' see page 44 see abstract	1,2,6,7
A	US,A,4 711 896 (J. BAR-TANA ET AL.) 8 December 1987 cited in the application see column 1 see claims 1-8	1-8
A	DIABETES vol. 37, 1988, pages 1618 - 1624 R. TZUR ET AL. 'Hypolipidemic, antiobesity, and hypoglycemic-hypoinsulinemic effects of beta,beta'-methyl-substituted hexadecanedioic acid in sand rats' cited in the application see the whole document	1-8

INTERNATIONAL SEARCH REPORT

International application No.
[redacted]/EP 93/01014

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although claims 4 and 8 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims searched completely: 2. Claims searched incompletely: 1,3-8.
Due to the large number of compounds which are theoretically defined by the markush formulas of claims 1,6 and 8, the search had to be restricted to the compounds explicitly mentioned in the description and the claims.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. EP 9301014
SA 73398

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 30/07/93
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-A-2234399	31-01-74	AT-B- 338434	25-08-77
		AU-A- 5812973	16-01-75
		BE-A- 802414	16-01-74
		CA-A- 1022849	20-12-77
		JP-A- 49085244	15-08-74
		NL-A- 7309851	21-01-74
US-A-4711896	08-12-87	DE-A- 3423166	02-01-86
		AU-A- 4607185	24-01-86
		CA-A- 1262552	31-10-89
		WO-A- 8600298	16-01-86
		EP-A,B 0185080	25-06-86
		JP-T- 61502537	06-11-86